American Journal of Transplantation 2007; 7: 2496–2503
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2007.01983.x

Individualized Mycophenolate Mofetil Dosing Based on Drug Exposure Significantly Improves Patient Outcomes After Renal Transplantation

Y. Le Meur[a,*], M. Büchler[b], A. Thierry[c], S. Caillard[d], F. Villemain[e], S. Lavaud[f], I. Etienne[g], P.-F. Westeel[h], B. H. de Ligny[i], L. Rostaing[j], E. Thervet[k], J. C. Szelag[a], J.-P. Rérolle[a], A. Rousseau[l], G. Touchard[c] and P. Marquet[m]

[a] *Department of Nephrology, University Hospital, Limoges, France*
[b] *Department of Nephrology, University Hospital, Tours, France*
[c] *Department of Nephrology, University Hospital, Poitiers, France*
[d] *Department of Nephrology, University Hospital, Strasbourg, France*
[e] *Department of Nephrology, University Hospital, Angers, France*
[f] *Department of Nephrology, University Hospital, Reims, France*
[g] *Department of Nephrology, University Hospital, Rouen, France*
[h] *Department of Nephrology, University Hospital, Amiens, France*
[i] *Department of Nephrology, University Hospital, Caen, France*
[j] *Department of Nephrology, University Hospital Rangueil, Toulouse, France*
[k] *Hôpital Necker, Paris, France*
[l] *Department of Biomathematics, Faculty of Pharmacy, Limoges, France*
[m] *Department of Pharmacology-Toxicology, University Hospital, Limoges, France*
* *Corresponding author: Yannick Le Meur, yannick.lemeur@chu-brest.fr*

Efficacy and safety of mycophenolate mofetil (MMF) may be optimized with individualized doses based on therapeutic monitoring of its active metabolite, mycophenolic acid (MPA). In this 12-month study, 137 renal allograft recipients from 11 French centers receiving basiliximab, cyclosporine A, MMF and corticosteroids were randomized to receive either concentration-controlled doses or fixed-dose MMF. A novel Bayesian estimator of MPA AUC based on three-point sampling was used to individualize doses on posttransplant days 7 and 14 and months 1, 3 and 6. The primary endpoint was treatment failure (death, graft loss, acute rejection and MMF discontinuation). Data from 65 patients/group were analyzed. At month 12, the concentration-controlled group had fewer treatment failures ($p = 0.03$) and acute rejection episodes ($p = 0.01$) with no differences in adverse event frequency. The MMF dose was higher in the concentration-controlled group at day 14 ($p < 0.0001$), month 1 ($p < 0.0001$) and month 3 ($p < 0.01$), as were median AUCs on day 14 (33.7 vs. 27.1 mg•h/L; $p = 0.0001$) and at month 1 (45.0 vs. 30.9 mg•h/L; $p < 0.0001$). Therapeutic MPA monitoring using a limited sampling strategy can reduce the risk of treatment failure and acute rejection in renal allograft recipients 12 months posttransplant with no increase in adverse events.

Key words: Area-under-curve, mycophenolate mofetil, renal transplantation, therapeutic drug monitoring

Received 13 April 2007, revised 3 August 2007 and accepted for publication 3 August 2007

Introduction

Extensive experience has supported the efficacy of fixed-dose (FD) mycophenolate mofetil (MMF) in combination with calcineurin inhibitors and corticosteroids for the prevention of acute rejection in patients receiving renal, cardiac or hepatic allografts (1). While doses of many immunosuppressants (cyclosporine A, tacrolimus, sirolimus, everolimus) are individualized using therapeutic drug monitoring (2), MMF was approved for clinical use in adult renal transplant recipients at a dose of 2 g/day. As clinical use evolved, however, it became clear that MMF possessed some of the same characteristics as other agents whose efficacy and safety are optimized with therapeutic drug monitoring; these characteristics include inter-patient variability (with exposures varying by 10-fold) and intra-patient variability over time (a 50–100% increase in exposure can occur over the first 3–6 months posttransplantation) (3). Because of its complex pharmacokinetics, many factors can influence mycophenolic acid (MPA) exposure, including kidney and liver function, levels of serum albumin, alterations in absorption and combination with other immunosuppressive agents (reviewed by van Gelder and Shaw) (4). In particular, concurrent administration of cyclosporine has been found to significantly lower MPA levels (5).

Furthermore, the current common practice of combining MMF with other agents in novel regimens such as low-dose calcineurin inhibitors, mTOR (mammalian target of rapamycin) inhibitors or steroid avoidance has allowed physicians to use 'a la carte' doses of these drugs; at the same time, however, it increases the risk of inadequate or excessive immunosuppression when used without therapeutic monitoring. Finally, this same issue of adequate dosing is also of concern when MMF is used for the induction and maintenance of remission of lupus nephritis in systemic lupus erythematosus (6).

MMF is an inactive prodrug that is converted to its active metabolite MPA by intestinal, liver and plasma esterases (3). MPA acts as an uncompetitive and reversible inhibitor of inosine-5'-monophosphate dehydrogenase, an enzyme necessary for lymphocyte mitosis (7). Post-licensing studies have demonstrated an association between clinical events and MPA plasma concentrations, with a stronger relationship for MPA area under the concentration-time curve (AUC) than for trough levels (8). An MMF concentration-controlled (CC) trial in renal transplant patients confirmed that the risk of acute rejection is higher in patients with low MPA exposure, and adverse event-related withdrawals are more frequent with high MPA exposure (9,10).

Because of the pharmacokinetic variability and strong association between clinical events and MPA AUCs (9, 10), individualized dosing based on MPA therapeutic monitoring in renal transplantation has been advocated, with recommended target AUCs between 30 and 60 mg•h/L (11–13). Still, most transplant centers have yet to implement monitoring programs, largely due to technical difficulties, to concern about the optimal methodology and to lack of definitive clinical data supporting efficacy. Single determinations of MPA concentrations have limited correlation with 12-h AUC measurements that are in and of themselves impractical for most centers. Recently, we developed an accurate and easily applied pharmacokinetic model for determining MPA exposure using a Bayesian estimator of MPA AUC that employs a three-point MPA concentration sampling strategy (14,15). The present study reports the results of the multicenter Adaptation de Posologie du MMF en Greffe Rénale (APOMYGRE) trial involving renal allograft recipients randomized to receive either FD MMF or a CC regimen in which MMF dose adjustments were calculated based on this model to reach predefined MPA target levels.

Materials and Methods

Study design

This 12-month, multicenter, open-label, randomized trial enrolled consecutive, eligible renal allograft recipients $\geq$18 years old receiving a first or second transplant at 11 centers in France (Clinical Trial Registry No. NCT0019967). Exclusion criteria included historic or current panel reactive antibodies >50%; a history of malignancy within 5 years (except for successfully treated squamous or basal cell carcinoma of the skin); pregnancy or women not using contraception and psychiatric or gastrointestinal disorders. Within the first 3 days posttransplant, patients were randomized by an interactive voice response system administered by a private company; randomization was balanced within centers in blocks of 4 patients, and patients were enrolled and assigned to one of the two groups by physicians at each center. Follow-up was 12 months. The study was approved by the regional ethics committee of Limoges, France and complied with the Declaration of Helsinki. All patients gave written informed consent. With an expected failure rate of 55% in the FD group and an expected reduction of 50% in the CC group, it was determined prior to the study that at least 57 patients per group were required to achieve a power of 0.80 at a significance level of 0.05; we planned to enroll 67 patients per group to allow for a 10–15% drop-out rate.

Study treatment

Patients were randomized 1:1 to receive CC or FD MMF in a quadruple immunosuppressive regimen that included i.v. basiliximab (20 mg on days 0 and 4), i.v. methylprednisolone (500 mg on day 0) and cyclosporine. Subsequently, prednisolone was given orally at 1 mg/kg/day on days 1–7, 0.5 mg/kg/day on days 8–14, then reduced weekly by 5 mg/day to a 20 mg total dose and then reduced by 2.5 mg/day weekly to 10 mg/day. After 1 month, prednisolone doses were reduced weekly by 2.5 mg/day until discontinued (if possible) and according to the center practice. Cyclosporine (8 ± 2 mg/kg/day) was commenced within 3 days posttransplant and adjusted to maintain cyclosporine 2-h post-dose (C2) levels of 1300–1500 ng/mL through week 4, 1100–1300 ng/mL months 2–3, 900–1100 ng/mL months 4–6 and 800 ng/mL months 7–12. All cytomegalovirus antibody-negative recipients receiving allografts from cytomegalovirus antibody-positive donors were given oral prophylaxis (valacyclovir or valgancyclovir) for 3 months. Trimethoprim-sulfamethoxazole was administered orally to all patients for *Pneumocystis* prophylaxis.

In both groups, patients initially received MMF at the dose of 1 g twice daily until day 7. MPA was measured by high-performance liquid chromatography (HPLC) with an ultraviolet detector on posttransplant days 7 and 14 and months 1, 3, 6 and 12. All laboratories participated in the Mycophenolate International Proficiency Testing Scheme of D. Holt (Analytical Services International, Ltd., London, UK). Overall, the linearity range was at least 0.5–20 mg/L (samples over 20 mg/L being diluted) and inter-assay imprecision and inaccuracy was less than 15% over the linearity range, except at the lower limit of quantitation where 20% were accepted. MPA AUC was calculated using Bayesian estimations specific for MMF and based on samplings at 20 min and at 1 and 3 h post-administration (15). In the CC group, MMF dose adjustments were calculated by a computer program (available at www.chu-limoges.fr/stp/stpacces.htm, June 21, 2007) to reach an MPA AUC target of 40 mg•h /L. The minimum dose change was 250 mg twice a day. Each dose adjustment of at least 250 mg twice a day that was able to result in an AUC closer to 40 mg•h /L was proposed by the program to the physician. The maximum allowed dose was 4 g/day. In the FD group, MMF dose adjustments based on clinical experience were permitted; MPA AUC data were withheld from physicians.

Acute rejection was diagnosed by renal biopsy except in patients with contraindications and were graded according to the Banff classification, in which case diagnosis was based on clinical and laboratory criteria (in particular, any unexplained increase in serum creatinine). Episodes of acute rejection were treated with i.v. corticosteroids; resistant episodes were treated with monoclonal antibodies (OKT3) or polyclonal antithymocyte antibodies.

Assessments and analyses

Treatment failure (a composite of death, graft loss, acute rejection and MMF discontinuation) was the primary end point. The secondary objectives were

to compare the incidence and the severity of acute clinically suspected and biopsy-proven acute rejection and the incidence of adverse events in the two groups. All adverse events were recorded irrespective of severity or relationship to the study medication, with special attention to the following: anemia (hemoglobin level <10 g/dL, excluding the first month post-transplant or evident blood loss); leukopenia (total white cell count $<3 \times 10^9$/mL); gastrointestinal adverse events (diarrhea, constipation, anorexia, abdominal pain, nausea or vomiting) and infections (cytomegalovirus, other viruses and other infections).

The primary efficacy analysis was based on the intention-to-treat (ITT) population, defined as all randomized patients who received study medication and who completed the day 7 visit (time of the first dose adjustment in the CC group). For the comparison between categorical data, we used the Pearson chi-square test. Contiguous data were tested for normal distribution using the Kolmogorov–Smirnow test. Normally distributed data were analyzed by the parametric *t*-test, whereas a nonparametric test (Mann–Whitney test) was used otherwise. Time to acute rejection was calculated as the time elapsed between grafting and the event, and was summarized by the Kaplan–Meier product limit estimator. A Cox proportional hazards regression model was used in the multivariate analysis to clarify whether the prognostic value of individual dose adjustment was independent of other potential prognostic indicators (e.g. recipient age, donor age, gender, HLA mismatch and cold ischemia time). The hazard rate ratios (HRR) of the Cox analysis were expressed as exp (β). Statistical analyses were performed using S-Plus 6 (Insightful Corporation, Seattle, WA).

Results

Baseline characteristics

Between September 2003 and October 2004, 137 patients were enrolled in the study (CC, n = 70; FD, n = 67). The mean recruitment was 12 patients (5–24 per center). There were seven withdrawals (CC, n = 5; FD, n = 2) due to death, primary nonfunctioning graft or because MMF was not administered. The ITT population included 65 patients in each group. Patient groups were similar in terms of age, number of second grafts, HLA mismatch, donor age and panel reactive antibody (PRA); all were considered at low immunological risk. The sex ratio differed between groups in that there were a larger percentage of males in the CC-treated group (Table 1).

Table 1: Demographic and background characteristics of patients and donors

	Concentration-controlled group	Fixed-dose group
Number of patients		
Enrolled	70	67
ITT population*	65	65
Age (years)	50 ± 14	49 ± 13
Female/male (%)	29/71	42/58
Second graft, number	3	0
Cold ischemia time (h)	17.6 ± 6.27	18.5 ± 6.17
HLA mismatches	3.74 ± 1.14	3.77 ± 1.11
Donor age (years)	49 ± 15	46 ± 14
Panel reactive antibodies, number		
0%	58	61
0–35%	7	4

* Seven early withdrawals for death, graft loss and deviation from the protocol before day 7.
Results expressed as mean ± SD unless otherwise indicated.
HLA, human leukocyte antigen; ITT, intention-to-treat.

Immunosuppressant dose and exposure

Recommended MMF dose adjustments in the CC group were instituted in 85% of patients; noncompliance was due to adverse events in 7% of patients, with the remainder due to physician's inattention or errors. At months 1 and 3, 82% and 51%, respectively, of patients in the CC group were receiving MMF doses in excess of 2 g/day (Table 2). At month 6, 48% of the patients in the CC group received doses below 2 g/day.

Prior to the first MMF dose adjustment at day 7, only a minority of patients in either group had MPA AUC levels that reached the minimum therapeutic level of 30 mg•h/L (Table 3). By day 14, median MPA exposure was significantly higher in the CC group than in the FD group, with a majority of patients in the CC group, but not in the FD group, having met targeted levels. At month 1, the CC group again had significantly higher median MPA AUC, with more than 90% of patients achieving target levels. A small but variable proportion of patients (5–21% over the course of the study) had MPA AUC >60 mg•h/L. Cyclosporine C2 levels were similar between groups (Table 4) and were consistent with predefined target levels, declining from approximately 1400 ng/mL at day 14 to approximately 750 ng/mL at month 12.

Efficacy and safety outcomes

There was a significantly lower incidence of treatment failure, the primary end point, in the CC group compared to the FD group (29.2% vs. 47.7%, p = 0.03; Table 5). This reduction can exclusively be attributed to fewer patients experiencing acute rejection episodes: clinical rejection was reported in 8 (12.3%) patients in the CC-treated group versus 20 (30.7%) in the FD-treated group (p = 0.01), and biopsy-proven acute rejection was reported in 5 (7.7%) and 16 (24.6%) patients in each group, respectively (p = 0.01) (Table 5). Of these episodes, only 2/8 (25%) were grade II in the CC-treated group compared with 7/20 (35%) in the FD-treated group. During the first year posttransplant, the cumulative incidence of acute rejection episodes was significantly reduced in the CC group (Figure 1). Cox analysis confirmed the efficacy of the tested individualized dose adjustment strategy versus the FD strategy. The group factor (adjusted vs. FD) was the most powerful indicator of acute rejection (HRR = 1.67, p = 0.017). The other variables were not significant predictive factors of acute rejection. After deletion of the nonsignificant variables, a new Cox analysis was performed that showed that only the group factor was a significant predictor of acute rejection (HRR = 1.65; CI 95% = 1.09, 2.54; p = 0.02).

Of the 10 episodes of acute rejection occurring in the first 3 months posttransplant, seven were associated with an MPA AUC value <30 mg•h/L while three were associated

Table 2: Comparisons of mycophenolate mofetil (MMF) dosage between concentration-controlled (CC) and fixed-dose (FD) groups

	Day 7	Day 14	Month 1	Month 3	Month 6	Month 12
Mean ± SD dose (mg/d)						
CC	2000	2698 ± 543	2969 ± 780	2279 ± 878	1924 ± 770	1827 ± 654
FD	2000	2000	1960 ± 186	1852 ± 441	1778 ± 466	1958 ± 306
p-value		<0.0001	<0.0001	<0.01	0.222	0.206
MMF dose distribution in the CC group (%)						
<2 g		8	6	26	48	44
2 g		12	12	23	22	36
2.5–3 g		80	42	36	25	20
3.5–4 g		0	40	15	5	0
MMF dose distribution in the FD group (%)						
2g		100	95	84	79	90.0
1g–1.5g		0	5	12	16	10.0
<1g		0	0	4	5	0

with a value between 30 and 45 mg•h/L (Table 6). There were no episodes associated with an AUC >45 mg•h/L. This was no longer true after 3 months, with 7 out of 18 late rejection episodes associated with MPA AUC >45 mg•h/L. Death, graft loss and MMF discontinuation were similar between groups, as were overall survival (98% for both groups) and graft survival (97% vs. 98% for the CC vs. FD groups, respectively). The steroid withdrawal was successful in 83% of patients in both groups.

Nearly every patient reported one or more adverse events (90% in the CC group and 97% in the FD group). The most commonly reported events were infections, anemia, gastrointestinal events and leukopenia (Table 7). The incidence of these adverse events was similar between the two groups. In particular, there was no difference in the incidence or severity of cytomegalovirus infections or bacterial infections. The only notable exception was the higher incidence of herpes virus infections in the CC group (Table 7). No association was found between MPA exposure and the occurrence of adverse events.

Laboratory assessments performed on days 7 and 14 and at months 1, 3, 6 and 12 showed no difference between the two groups in mean leukocyte counts, hemoglobin levels or proteinuria (Table 4). At 12 months, there was a trend toward better renal function in the CC group compared with the FD group, with lower serum creatinine levels (137 ± 45 vs. 150 ± 56 μmol/L; p = 0.15) and greater creatinine clearance (56.66 ± 21.6 vs. 52.88 ± 16.5 mL/min; p = 0.27).

Discussion

This study has demonstrated that adjusting MMF doses in response to MPA AUC determinations allows patients to achieve therapeutic MPA levels faster than patients receiving FD of MMF. The majority of patients in the CC group required doses in excess of 2 g/day (many as high as 4 g/day) to achieve therapeutic levels in the early months posttransplant, which, in turn, led to a reduction in treatment failure, the primary efficacy end point and the CC group compared with the FD group without an increased risk of MMF-associated adverse events. Yet by 6 months, there were no significant differences in dose or MPA exposure between the two groups, reflecting the combined effects of dose reductions in the CC group and the

Table 3: Comparisons of mycophenolic acid (MPA) area under the concentration-time curve (AUC) between concentration-controlled (CC) and fixed-dose (FD) groups

	Day 7	Day 14	Month 1	Month 3	Month 6	Month 12
Median and range AUC (mg•h/L)						
CC	24.7	33.7	45.0	43.6	37.2	36.8
	6.7–72.0	11.8–72.8	19.9–98.0	5.6–87.7	9.3–67.3	8.6–79.0
FD	25.4	27.1	30.9	37.5	33.1	42.0
	8.8–149.3	15.5–68.8	13.2–125.2	7.7–99.7	9.1–77.5	10.3–116.1
p-value	0.9685	<0.0001	<0.0001	0.0712	0.1039	0.4048
Proportion with MPA AUC >30 mg•h/L (%)						
CC	20.6	68.3	90.8	80.3	74.5	72.7
FD	29.0	30.2	55.5	66.6	57.8	70.3
Proportion with MPA AUC >60 mg•h/L (%)						
CC	3.1	1.6	13.8	21.3	8.4	7.2
FD	3.2	1.6	4.7	11.1	5.2	12.5

Table 4: Cyclosporine concentrations and laboratory results in the concentration-controlled (CC) and fixed-dose (FD) groups

	D14	M1	M3	M6	M12
CsA C2 level (ng/mL)					
CC	1453 ± 385	1431 ± 415	1117 ± 304	933 ± 243	743 ± 250
FD	1393 ± 435	1376 ± 367	1036 ± 278	950 ± 298	754 ± 230
Leukocyte (10^9/mL)					
CC	11.47 ± 3.10	7.87 ± 2.50	6.10 ± 2.20	6.53 ± 2.04	6.86 ± 2.96
FD	11.03 ± 3.10	7.73 ± 2.40	6.24 ± 2.60	5.76 ± 1.70	6.13 ± 2.44
Hemoglobin (g/dL)					
CC	10.4 ± 1.7	11.3 ± 1.4	11.9 ± 1.8	12.2 ± 1.8	12.9 ± 1.8
FD	10.5 ± 1.6	11.6 ± 1.5	12.0 ± 1.7	12.2 ± 1.7	12.4 ± 1.5
Serum creatinine level (μmol/l)					
CC	175 ± 119	142 ± 55	136 ± 40	132 ± 34	137 ± 45
FD	177 ± 115	152 ± 65	149 ± 47	148 ± 53	150 ± 56
Proteinuria (mg/day)					
CC	490 ± 548	341 ± 506	247 ± 287	212 ± 375	190 ± 259
FD	566 ± 486	340 ± 271	192 ± 172	175 ± 168	233 ± 533

Results are expressed as mean ± SD.
CsA C2, cyclosporine 2-h post-dose; D, day; M, month.

previously described suboptimal MPA exposure occurring in patients receiving FD MMF in the early posttransplant period (3,16). Of note, unlike the FDCC (FD vs. CC) MMF trial (17), which showed no improvements in outcomes with CC MMF dosing, our study had a high rate of physician compliance (85%) in instituting recommended MPA dose adjustments (17).

Despite the fact that in the present study corticosteroids were withdrawn between 4 and 6 months in most patients and target levels for cyclosporine were relatively low, the risk of biopsy-proven acute rejection and clinical acute rejection in the FD group (31%) was still comparable to published reports using a similar immunosuppressive regimen immediately posttransplant (18–20). Immunosuppressant minimization perhaps contributed to episodes of late (after 4 months) acute rejection, mainly in the FD group. Although the study design did not require that AUC be measured at the time of acute rejection, retrospective assessment of the MPA AUC value measured at the time point nearest to the event showed that no episodes occurred when the MPA AUC was >45 mg•h/L. Together with data demonstrating the low cumulative incidence of acute rejection (12%) in the CC group, these findings suggest that cyclosporine minimization and steroid withdrawal can safely be achieved in many patients by monitoring MPA exposure. Recommended therapeutic window for MPA AUC has been derived from the RCCT study in renal patients under cyclosporine A, MMF and steroids (10). This recommendation has been secondarily validated by Kiberd showing that an AUC <30 mg•h/L would correctly identify 79% of patients rejection within 3 months (21). These two studies used standard dose of Cyclosporine and steroids. In minimization protocols, however, higher target for MPA AUC has been proposed. In the Caesar trial for example, groups with cyclosporine A reduction or withdrawal required higher MPA exposure to prevent acute rejection than the

Table 5: Comparisons of efficacy outcomes between treatment groups

	Concentration-controlled group (n = 65)	Fixed-dose group (n = 65)	p-Value
Treatment failure (%)	19 (29.2)	31 (47.7)	0.03
Death	1	1	NS
Graft loss	1	0	NS
MMF discontinuation	9	10	NS
Any acute rejection (%)	8 (12.3)	20 (30.7)	0.01
Biopsy-proven acute rejection (%)	5 (7.7)	16 (24.6)	0.01
Banff classification	Grade I: 3 Grade II: 2	Grade I: 9 Grade II: 7	

MMF, mycophenolate mofetil; NS, not significant.

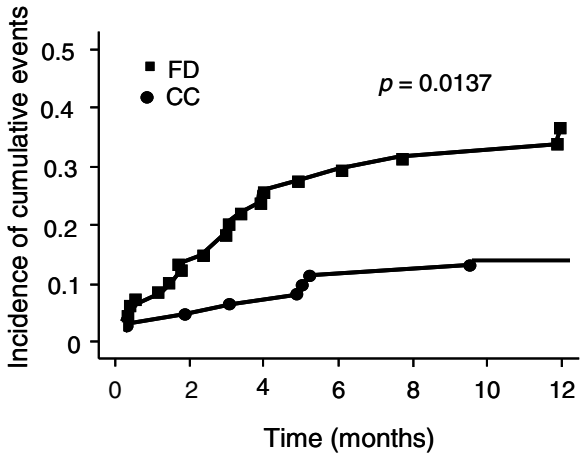

Figure 1: The cumulative incidence of acute rejection episodes during the first year posttransplant. CC, concentration-controlled; FD, fixed dose.

Table 6: Area under the concentration-time curve (AUC) and incidence of acute rejection before month 3

AUC (mg•h/L)	Number of acute rejection episodes	Number of AUCs	Percent AUCs associated with acute rejection
<30	7	192	3.6
30–45	3	126	2.4
>45	0	61	0

The MPA AUC closest to the event was compared with the AUC not associated with an acute rejection episode.

group with standard dose of cyclosporine (13, 22). More recently, according to the preliminary results of an ongoing study, de Fijter proposed a target as high as 75 mg•h/L to allow cyclosporine withdrawal after 6 months in patients under a triple therapy (23). Although our study was not designed to determine precise therapeutic levels, 45 mg•h/L seems a reasonable target based on our data.

The variability in MPA exposure following the administration of MMF observed in this study and previously reported by other investigators is a result of its complex pharmacokinetics. In the early posttransplant period, MPA AUC in renal allograft recipients is positively predicted by levels of serum creatinine and serum albumin (24), reflecting the impact of renal function and protein binding on MPA clearance. Another potential contributor to variability is enterohepatic recirculation, in which MPA is converted to MPA glucuronide (MPAG), an inactive metabolite that is eliminated in bile and urine (25). Intestinal deglucuronidation converts MPAG back to MPA, which is subsequently reabsorbed and results in a second MPA AUC peak (3,26). Cyclosporine decreases biliary excretion of MPAG (27), which interferes with enterohepatic recirculation and lowers MPA exposure; the mechanism is believed to involve the inhibition of multidrug resistance-associated protein 2 in hepatocytes (28). Thus, patients receiving cyclosporine tend to have lower MPA exposures than those receiving other agents (28–30).

In the current study, many patients in the CC group received much higher doses of MMF than those in the FD group without an increased occurrence of adverse events. A possible explanation may be that there were insufficient numbers of patients to identify significant differences between groups in adverse events. Other studies attempting to correlate MPA exposure with adverse events have also yielded inconsistent findings; however, one study reported a correlation between adverse events and MPA AUC and C_{30} (30-min post-dose) MPA levels (31), while others found a relationship between free MPA levels and hematological toxicity (32,33). A correlation between acyl-MPAG (an MPAG metabolite) levels and anemia has been observed, with the acyl-MPAG/MPA ratio highly significant, but no correlation between anemia and free MPA levels was reported in patients receiving tacrolimus/MMF (34). A correlation between acyl-MPAG and gastrointestinal side effects also has been reported (35). The lack of consistent correlations between MPA levels and adverse events may reflect the nature of the events, which have multiple causes, and may be further complicated by the fact that small numbers of patients were evaluated in many of these studies as well. A recent review of pivotal study data that included nearly 1000 patients reported no difference in the proportion of patients able to tolerate 3 g/day versus 2 g/day of MMF in the first 2 weeks posttransplant (13).

Despite the advantages of using a CC regimen as we have demonstrated in this study, MPA monitoring is not yet widely accepted due to the complexities of MPA pharmacokinetics, lack of accurate measurement tools and MPA AUC calculations. Two methods have been employed to estimate inter-dose AUCs using a limited sampling

Table 7: Summary of adverse events (AE)

	Concentration-controlled group (n = 65)		Fixed-dose group (n = 65)	
	Events	Patients (%)	Events	Patients (%)
Total AE	219	63 (97)	209	59 (90)
Gastrointestinal events	27	16 (25)	24	13 (20)
Anemia	57	43 (66)	53	40 (61)
Leukopenia	34	25 (40)	32	22 (34)
Infection	101	50 (77)	100	48 (74)
Cytomegalovirus	23	21 (32)	27	21 (32)
Syndrome	7		7	
Tissue-invasive disease	5		4	
Antigenemia and/or PCR	11		16	
Other viruses	33	21 (32)	22	
Herpes	8*		1	
Nonherpes	25		21	16 (25)
Bacterial infection	43	29 (45)	49	28 (43)
Other infections	2	2 (3)	2	2 (3)

*$p < 0.05$.
PCR, polymerase chain reaction.

strategy. The first, based on multilinear regression models, requires strict adherence to sampling times for calculation of MPA $AUC_{0–12h}$ (36). The second, known as Bayesian forecasting, estimates individual pharmacokinetic parameters based on experience with similar patients and involves more complex calculations but is more precise and allows flexibility in sampling times. Our recent publication presenting a pharmacokinetic model for MPA AUC that accounts for enterohepatic recirculation (14) was the basis for the development of the Bayesian estimator used in this study; this Bayesian estimator has a bias less than 10% in estimating MPA $AUC_{0–12h}$ (15). MPA is assayed using a commercial enzyme-multiplied immunoassay technique (EMIT) or HPLC. Although convenient, the EMIT assay overestimates MPA concentrations by as much as 50% due to cross-reactivity with certain MPA metabolites (37); HPLC is more accurate but not widely available clinically. The final concern limiting the widespread use of MPA monitoring is the perception that it increases drug-associated costs. To address this issue, an ongoing pharmacoeconomic substudy of the present trial has been designed to evaluate the cost-effectiveness of MPA monitoring.

In conclusion, our study demonstrated that MMF dose individualization using a Bayesian estimator is feasible, effective and safe in renal transplant patients. These results can be extrapolated to routine practice in low-risk renal transplant recipients receiving MMF in conjunction with induction therapy, corticosteroids and cyclosporine. The benefits in low-risk patients suggest that MMF therapeutic monitoring is likely to provide similar or even greater benefits in higher-risk patients. Such patients may include those receiving a second allograft or patients on alternate immunosuppressive regimens (e.g. no induction, calcineurin inhibitor-sparing or corticosteroid-free regimens). Whether the above mentioned suggestion can be extended to patients treated with tacrolimus or sirolimus needs further investigation. In this situation, more patients would be expected to be in the MPA AUC 'therapeutic' range in the early posttransplant period, so the utility of MMF TDM in the low risk population should be evaluated. Nevertheless in high-risk patients under TAC regimen or in corticosteroid-free regimens or in tacrolimus minimization strategy TDM of MMF could be a useful tool as well.

Finally, our results demonstrating significant reductions in treatment failure in the CC group under conditions of steroid withdrawal and low-target levels of cyclosporine suggest that MPA monitoring might contribute substantially to the success of immunosuppressive minimization protocols.

Acknowledgments

We would like to acknowledge the collaboration and commitment of all the local investigators: Y. Lebranchu, Tours; O. Toupance, Reims; M. Godin, Rouen; J.M. Goujon, Poitiers; B. Moulin, Strasbourg and B. Hurault de Ligny, Caen and all the local laboratory scientists responsible for MPA therapeutic drug monitoring: B. Diquet, Angers; P. Compagnon, Rouen; D. Debruyne, Caen; L. Harry, Amiens; G. Hoizey, Reims; E. Jacqz-Aigrain, Paris; C. Loichot, Strasbourg; N. Venisse, Poitiers; S. Saivin, Toulouse and F. Saint-Marcoux, Limoges. Technical help from F. Yagoubi and V. Leprivey was very much appreciated. This study was funded by the Regional Clinical Research Program of Limoges University Hospital, l'Agence Française de Sécurité Sanitaire et des Produits de Santé and Roche France.

References

1. Ciancio G, Miller J, Gonwa TA. Review of major clinical trials with mycophenolate mofetil in renal transplantation. Transplantation 2005; 80(2 Suppl): S191–S200.
2. Kahan BD, Keown P, Levy GA, Johnston A. Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. Clin Ther 2002; 24: 330–350.
3. Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet 1998; 34: 429–455.
4. van Gelder T, Shaw LM. The rationale for and limitations of therapeutic drug monitoring for mycophenolate mofetil in transplantation. Transplantation 2005; 80(2 Suppl): S244–S253.
5. Smak Gregoor PJ, de Sevaux RG, Hene RJ et al. Effect of cyclosporine on mycophenolic acid trough levels in kidney transplant recipients. Transplantation 1999; 68: 1603–1606.
6. Ginzler EM, Dooley MA, Aranow C et al. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. N Engl J Med 2005; 24: 2219–2228.
7. Eugui EM, Allison AC. Immunosuppressive activity of mycophenolate mofetil. Ann N Y Acad Sci 1993; 685: 309–329.
8. Shaw LM, Korecka M, Venkataramanan R, Goldberg L, Bloom R, Brayman KL. Mycophenolic acid pharmacodynamics and pharmacokinetics provide a basis for rational monitoring strategies. Am J Transplant 2003; 3: 534–542.
9. Hale MD, Nicholls AJ, Bullingham RE et al. The pharmacokinetic-pharmacodynamic relationship for mycophenolate mofetil in renal transplantation. Clin Pharmacol Ther 1998; 64: 672–683.
10. van Gelder T, Hilbrands LB, Vanrenterghem Y et al. A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. Transplantation 1999; 68: 261–266.
11. Shaw LM, Nicholls A, Hale M, Meiser B, van Gelder T. Therapeutic monitoring of mycophenolic acid. A consensus panel report. Clin Biochem 1998; 31: 317–322.
12. Shaw LM, Holt DW, Oellerich M, Meiser B, van Gelder T. Current issues in therapeutic drug monitoring of mycophenolic acid: Report of a roundtable discussion. Ther Drug Monit 2001; 23: 305–315.
13. van Gelder T, Le Meur Y, Shaw LM et al. Therapeutic drug monitoring of mycophenolate mofetil in transplantation. Ther Drug Monit 2006; 28:145–154.
14. Premaud A, Debord J, Rousseau A et al. A double absorption-phase model adequately describes mycophenolic acid plasma profiles in de novo renal transplant recipients given oral mycophenolate mofetil. Clin Pharmacokinet 2005; 44: 837–847.
15. Premaud A, Le Meur Y, Debord J et al. Maximum a posteriori bayesian estimation of mycophenolic acid pharmacokinetics in renal transplant recipients at different postgrafting periods. Ther Drug Monit 2005; 27: 354–361.
16. Weber LT, Lamersdorf T, Shipkova M et al. Area under the plasma concentration-time curve for total, but not for free, mycophenolic acid increases in the stable phase after renal transplantation: A longitudinal study in pediatric patients. German Study Group on

Mycophenolate Mofetil Therapy in Pediatric Renal Transplant Recipients. Ther Drug Monit 1999; 21: 498–506.
17. van Gelder T, Tedesco-Silva H, de Fijter H et al. An open, prospective, randomized, controlled, multicenter study comparing fixed dose vs. concentration controlled mycophenolate mofetil regimens for de novo patients following renal transplantation (the FDCC trial)[abstract]. Am J Transplant 2006: 6(Suppl 12): 343.
18. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U.S. Renal Transplant Mycophenolate Mofetil Study Group. Transplantation 1995; 60: 225–232.
19. Schena FP, Vincenti F, Paraskevas S, Hauser I, Grinyo J. 12-month results of a prospective, randomized trial of steroid avoidance, steroid withdrawal and standard steroids in de novo renal transplant patients receiving cyclosporine, enteric-coated mycophenolate sodium (EC-MPS, myfortic (R)) and basiliximab [abstract]. Transplantation 2006; 82(Suppl 3): 84–85.
20. Ekberg H, Tedesco-Silva H, Demirbas A et al. Symphony—comparing standard immunosuppression to low-dose cyclosporine, tacrolimus or sirolimus in combination with MMF, daclizumab and corticosteroids in renal transplantation [abstract]. Transplantation 2006; 82(Suppl 3): 83.
21. Kiberd BA, Lawen J, Fraser AD, Keough-Ryan T, Belitsky P. Early adequate mycophenolic acide exposure is associated with less rejection in kidney transplantation. Am J Transplant 2004; 4:1079–1083.
22. Ekberg H, Grinyó J, Nashan B et al. Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: The CAESAR Study. Am J Transplant 2007; 7: 560–570.
23. de Fijter JW, Mourer JS, den Hartigh J, Mallat MJ, Berger SP. Concentration-controlled systemic exposure provides improved safety after CNI or MMF withdrawal [abstract]. Am J Transplant 2007; 7(Suppl 2): 208
24. Johnson AG, Rigby RJ, Taylor PJ et al. The kinetics of mycophenolic acid and its glucuronide metabolite in adult kidney transplant recipients. Clin Pharmacol Ther 1999; 66: 492–500.
25. Fulton B, Markham A. Mycophenolate mofetil. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in renal transplantation. Drugs 1996; 51: 278–298.
26. Shaw LM, Sollinger HW, Halloran P et al. Mycophenolate mofetil: A report of the consensus panel. Ther Drug Monit 1995; 17: 690–699.
27. Deters M, Kirchner G, Koal T, Resch K, Kaever V. Influence of cyclosporine on the serum concentration and biliary excretion of mycophenolic acid and 7-O-mycophenolic acid glucuronide. Ther Drug Monit 2005; 27: 132–138.
28. Hesselink DA, van Hest RM, Mathot RA et al. Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. Am J Transplant 2005; 5: 987–994.
29. Buchler M, Lebranchu Y, Beneton M et al. Higher exposure to mycophenolic acid with sirolimus than with cyclosporine cotreatment. Clin Pharmacol Ther 2005; 78: 34–42.
30. van Gelder T, Klupp J, Barten MJ, Christians U, Morris RE. Comparison of the effects of tacrolimus and cyclosporine on the pharmacokinetics of mycophenolic acid. Ther Drug Monit 2001; 23: 119–128.
31. Mourad M, Malaise J, Chaib Eddour D et al. Correlation of mycophenolic acid pharmacokinetic parameters with side effects in kidney transplant patients treated with mycophenolate mofetil. Clin Chem 2001; 47: 88–94.
32. Weber LT, Shipkova M, Armstrong VW et al. The pharmacokinetic-pharmacodynamic relationship for total and free mycophenolic acid in pediatric renal transplant recipients: A report of the German Study Group on Mycophenolate Mofetil Therapy. J Am Soc Nephrol 2002; 13: 759–768.
33. Heller T, Armstrong VW, Brandhorst G, Streit F, van Gelder T, Oellerich M. Relationship between free mycophenolic acid and hematologic side effects: Interim results from the FDCC study [abstract]. Transplantation 2006; 15: 82(1 Suppl 2): 344.
34. Kuypers DR, Vanrenterghem Y, Squifflet JP et al. Twelve-month evaluation of the clinical pharmacokinetics of total and free mycophenolic acid and its glucuronide metabolites in renal allograft recipients on low dose tacrolimus in combination with mycophenolate mofetil. Ther Drug Monit 2003; 25: 609–622.
35. Heller T, Armstrong VW, Brandhorst G, Englemayer J, van Gelder T, Oellerich M. Plasma concentrations of mycophenolic acid acyl glucuronide related to diarrhoea: Interim results from the FDCC study [abstract]. Transplantation 2006; 82(1 Suppl 2): 344–345.
36. Le Guellec C, Buchler M, Giraudeau B et al. Simultaneous estimation of cyclosporin and mycophenolic acid areas under the curve in stable renal transplant patients using a limited sampling strategy. Eur J Clin Pharmacol 2002; 57: 805–811.
37. Premaud A, Rousseau A, Le Meur Y, Lachatre G, Marguet P. Comparison of liquid chromatography-tandem mass spectrometry with a commercial enzyme-multiplied immunoassay for the determination of plasma MPA in renal transplant recipients and consequences for therapeutic drug monitoring. Ther Drug Monit 2004; 26: 609–619.